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Development and testing of a new disposable sterile device for labelling white blood cells

A. SIGNORE [1, 2], A. W. J. M. GLAUDEMANS [2], G. MALVIYA [1, 2], E. LAZZERI [3]
N. PRANDINI [4], A. L. VIGLIETTI [5], E. F. J. DE VRIES [2], R. A. J. O. DIERCKX [2]

[1]*Nuclear Medicine Unit, Faculty of Medicine and Psychology "La Sapienza" University, Rome, Italy*
[2]*Department of Nuclear Medicine and Molecular Imaging University Medical Center Groningen University of Groningen, Groningen, the Netherlands*
[3]*Regional Center of Nuclear Medicine University of Pisa, Pisa, Italy*
[4]*Department of Nuclear Medicine, Azienda Ospedaliero-Universitaria Ferrara, Ferrara, Italy*
[5]*Department of Nuclear Medicine Ospedale S. Croce e Carle, Cuneo, Italy*

***Aim*. White blood cell (WBC) labelling requires isolation of cells from patient's blood under sterile conditions using sterile materials, buffers and disposables under good manufacturing practice (GMP) conditions. Till now, this limited the use of white blood cell scintigraphy (WBC-S) only to well equipped laboratories with trained personnel. We invented, developed and tested a disposable, sterile, closed device for blood manipulation, WBC purification and radionuclide labelling without exposing patient's blood and the operator to contamination risks. This device prototype and a final industrialized device (Leukokit®) were tested for WBC labelling and compared to standard procedure. Leukokit® was also tested in an international multi-centre study for easiness of WBC purification and labelling.**

***Methods*. On the device prototype we tested in parallel, with blood samples from 7 volunteers, the labelling procedure compared to the standard procedure of the International Society of Radiolabeled Blood Elements (ISORBE) consensus protocol with respect to cell recovery, labelling efficiency (LE), cell viability (Trypan Blue test) and sterility (haemoculture). On the final Leukokit® we tested the biocompatibility of all components, and again the LE, erythro-sedimentation rate, cell viability, sterility and apyrogenicity. ACD-A, HES and PBS provided by Leukokit® were also compared to Heparin, Dextran and autologous plasma, respectively. In 4 samples, we tested the chemotactic activity of purified WBC against 1 mg/ml of lipopolysaccharide (LPS) and chemotaxis of ^{99m}Tc-HMPAO-labelled WBC (925 MBq) was compared to that of unlabelled cells. For the multi-centre study, 70 labellings were performed with the Leukokit® by 9 expert operators and 3 beginners from five centers using blood from both patients and volunteers. Finally, Media-Fill tests were performed by 3 operators on two different days (11 procedures) by replacing blood and kit reagents with bacterial culture media (Tryptic Soy Broth) and testing sterility of aliquots of the medium at the end of procedure.**

***Results*. Tests performed with the prototype showed no significant differences with the standard procedure but a faster and safer approach. Tests performed with the final Leukokit® confirmed full biocompatibility, sterility and apyrogenicity of all reagents and plastic ware. Average WBC recovery with Leukokit® was comparable to that of the ISORBE protocol ($117x10^6 \pm 24x10^6$ *vs.* $132x10^6 \pm 29x10^6$ cells, P=not significant). No differences in red blood cells and platelet content were observed. LE was 82% ± 3% for Leukokit® and 65±5% for control (P=0.0003) being PBS vs autologous plasma the main reason of such difference. Cell viability was always >99.9% in both conditions. Chemotactic tests showed no differences between all Leukokit® samples and controls. Haemocultures and Media-Fill tests were always sterile. The procedure**

Corresponding author: Prof. dr. A. Signore, MD, PhD, Department of Nuclear Medicine, S. Andrea Hospital, La Sapienza University of Rome, Via di Grottarossa 1035, 00189 Rome, Italy.
E-mail: alberto.signore@uniroma1.it

was well accepted by expert operators and beginners, with a very fast learning curve (confidence after 2±2 labellings).

***Conclusion.* The invented device offers high level of protection to operators and patients. The derived Leukokit® is safe and easy to use, and gives a high LE of WBC without affecting cell viability and function. Being a registered closed, sterile medical device, it may allow easier and faster WBC labelling that is not limited to only well equipped laboratories. Also simultaneously labelling of multiple patients is possible.**

Key words: Leukocytes - Radionuclide imaging - Infection.

Radiolabelled white blood cell (WBC) scintigraphy is considered by most the "gold standard" nuclear medicine technique for imaging of infections,[1-5] with only a few exceptions, such as spondylodiscitis.[6, 7] WBC scintigraphy (WBC-S) is based on the *ex vivo* labelling, with ^{99m}Tc-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) or ^{111}Indium-oxine, of purified autologous white blood cells followed by intravenous reinjection into the patient. WBC labelling is considered a laborious and time-consuming technique. Because the final radiopharmaceutical cannot be sterilized and handling of potentially contaminated blood products is involved, WBC labelling exposes the patient as well as the operator to several potential risks. This implies that the whole procedure should be performed by trained personnel under strict regulations.[8] To this purpose there are different recommendations in different countries, although most prescribe that WBC labelling should be performed using an isolator or under a Class-A laminar flow cabinet in a Class-B or Class-C environment. It is also recommended to perform one cell labelling at a time (one patient) in order to avoid cross-contaminations. This recommendation further increases costs and time for performing WBC-S. Nevertheless, WBC-S is used all over the world, with few exceptions, for the diagnosis of sites of occult infection, showing a pivotal role in the diagnosis and follow-up of bone and vascular prostheses, osteomyelitis, inflammatory bowel diseases, fever of unknown origin and other disorders.[9, 10] The current protocol for white blood cell labelling is performed *ex vivo* with several steps in sequence: blood sample withdrawal, red blood cell sedimentation, removal of leukocyte-rich plasma, centrifugation and separation of white blood cells from plasma and platelets. After these preliminary steps, purified white blood cells are labelled with ^{99m}Tc-HMPAO or ^{111}In-oxine and unbound radioactive tracer is removed from the labelled cells by one or two additional centrifugations, before reinjection of the suspension into the patient.

An alternative technique to label WBC *in vivo* is based on the use of antigranulocyte murine monoclonal antibodies (mAb), or Fab' fragments, labelled with ^{99m}Tc. Delivered as a single-injection technique these alternatives eliminate the need for handling blood and the complex separation and labelling techniques that are required for WBC imaging. The diagnostic accuracy of radiolabelled mAb as compared to WBC-S is still a matter of debate and in most papers found to be lower than the accuracy of the *in vitro* labeling of leukocytes.[7] Therefore, most centers still consider the use of WBC as the "gold standard" for imaging infections.

To overcome the most important limitations of WBC labelling, a simplified labelling procedure that provides protection for operators and patients and that could be easily and safely applied simultaneously to several patients, would be extremely helpful. Therefore, one of us (AS) invented and patented a sterile single-use closed device for the introduction, handling, labelling and withdrawal of solvents, liquids and cells under sterile conditions and without changing internal pressures. Approximately a year after patenting, an Italian company (GIpharma srl) acquired all rights to industrialize the device and started its development and registration as medical device (Figures 1, 2).

In this study, a device prototype and the commercially available version of it, Leukokit®, were validated for the ex-vivo labelling of leukocytes with ^{99m}Tc-HMPAO. To this purpose, WBC labelling with the device was compared to the method described by the ISORBE consensus protocol.[8] Labelling efficiency (LE), cell viability and functionality (chemotaxis) after labelling were also evaluated. We tested the sterility of the labelling device and provided reagents, as well as the sterility of the WBC suspension after labelling. The easiness of use of the Leukokit® for both experienced operators and beginners was also evaluated in a multi-centre study.

Materials and methods

The first device

The first device was conceived as a modified Falcon tube with an inlet rubber cap, an outer needle

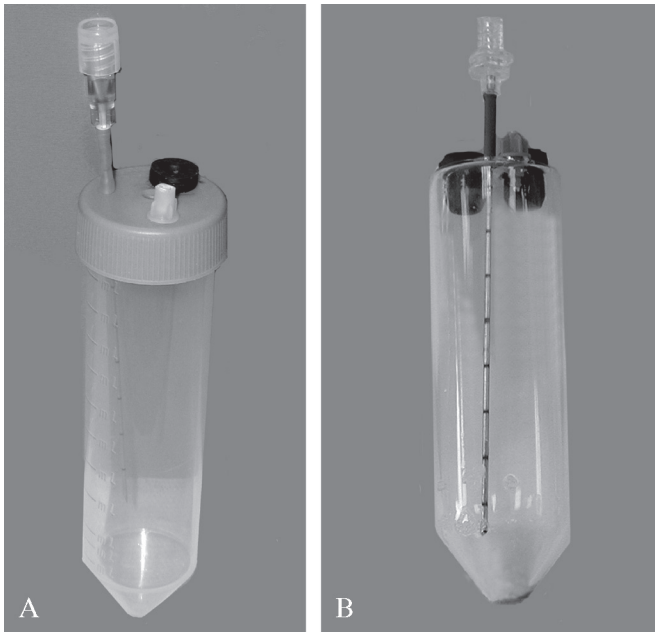

Figure 1.—The original prototypes of the sterile closed device for WBC labelling. A) The first patented prototype; B) the second prototype used for initial validation experiments.

of adjustable length and a filter to avoid modification of internal pressure. The first prototype was produced as a 35 mL Falcon tube (Figure 1A) to fit into most centrifuges and was patented, but never produced in a sterile sealed form.

The second device

After patenting, a second prototype was produced as sterile closed glass hardware with similar inlet and outlet valves as the first device (Figure 1B). This second prototype was produced in a few units, autoclaved for sterility purposes and used for initial experiments and to validate the procedure.

The Leukokit®

The device has been commercialized with the name of Leukokit® (GI Pharma s.r.l., Italy). The latest experiments described in this manuscript were performed with this kit that provides the following sterile components:

— A sealed sterile vial (the patented device) with an injection port, an air-venting filter and a one-way sealing valve of adjustable length connected with a plastic needle inside (Figure 2). The injection port,

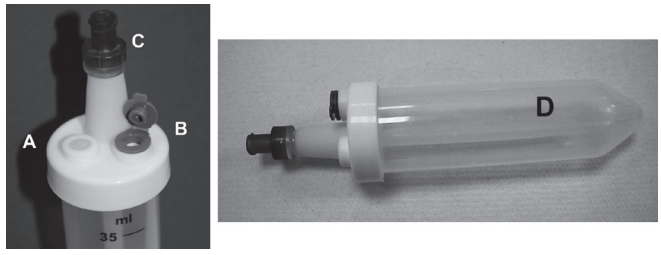

Figure 2.—The industrialized version of the device. A) injection port; B) air venting filter; C) one way outlet sealing valve of adjustable length; and D) plastic pipette inside the vial, connected to the adjustable outlet.

or inlet (port A), can be repeatedly pierced with needles and guarantees hermetic resealing after each withdrawal of the needle. This port is used to introduce the WBC rich plasma, the washing buffer PBS and the radiopharmaceutical for leukocyte labelling into the vial. The air-venting filter (port B) allows equilibration of air pressure between the inside and the outside of the vial whilst preserving sterility of the entering air. The one-way sealing valve, or outlet (port C), connected to a plastic pipette inside the vial, allows extraction of the solutions from the vial, preventing inadvertent introduction of air or liquids and resealing the vial following every syringe disconnection. The plastic pipette inside the separation vial allows accurate and progressive aspirations of the solutions from outside. Its elastic silicon supporting element allows to push down gradually the tip of the pipette inside the vial, thus allowing complete removal of supernatant without disturbing the cell pellet following centrifugations and to remove the labelled cells at the end of the procedure. The size of this device is compatible to fit into most of commercially available centrifuge rotors equipped for 50 ml Falcon tubes.

— A sedimentation syringe with a sealing valve. The syringe luer lock is sealed by its valve, thus providing a sterile closed environment. This special two-way valve can be opened only by connecting it to a female luer: i.e. to the vented spikes or the butterfly needle (also provided in the kit).

— A 10 ml vial of GMP produced, sterile anticoagulant reagent (acid citric dextrose, ACD-A; formulation consisting of an aqueous solution of anhydrous citric acid 7.3 g/L, sodium citrate dihydrate 22.0 g/l and dextrose monohydrate 24.5 g/L).

— A 10 ml vial of GMP produced, sterile, red blood cell sedimentation agent (10% High Molecular Weight 2-hydroxyethylstarch, HES).

— A 10 ml vial of GMP produced, sterile, isotonic, washing buffer (0.1 M phosphate buffered saline, pH 7.4, PBS).

— Two vented spikes, 2 butterfly needles 19-G and all luer-lock syringes needed for all labelling steps.

— Colour code stickers to identify samples from different patients.

Not supplied in the kit, but required for the procedure are: the radiopharmaceutical for the labelling of leukocytes (^{111}In-oxine or ^{99m}Tc-HMPAO or others), a centrifuge that is capable of spinning Falcon type tubes and radioprotective shields.

Therefore, Leukokit® represents a single-patient disposable sterile kit for WBC labelling with radioisotopes. All materials and components were tested for biocompatibility according to the appropriate UNI-EN-ISO-10993 and European Pharmaceutical Guidelines. The kit was tested for easiness of use in 70 subjects (60 patients and 10 volunteers) in five centers by 9 expert operators and 3 beginners. In seven subjects, the whole procedure was compared to the standard technique with respect to labelling efficiency (LE), cell viability (Trypan Blue exclusion test) and sterility (haemoculture).

Experiments performed with the devices

A withdrawal of 100 ml of peripheral blood using two 50 mL syringes filled with 9 mL of ACD-A each, as anti-coagulant, was collected from 7 volunteers. One syringe was processed under a Class-A laminar flow cabinet for WBC separation and labelling with ^{99m}Tc-HMPAO, using the published ISORBE protocol.[8] The second syringe was processed on the laboratory bench (unclassified room) using the sterile prototype of the patented vial and the same reagents as for the first sample. Briefly, 10 mL of 10% HES (Fresenius, France) was added for erythrocyte sedimentation and after 30 minutes the leukocyte-platelet-rich plasma (LPRP) was collected and centrifuged before labelling the cell pellet with 1 ml of ^{99m}Tc-HMPAO (740 MBq, 20 mCi). After 10 minutes, cells were washed with 5 mL autologous cell-free plasma (CFP) and then resuspended in 5 mL of CFP. On the final product, we calculated the LE and, on small aliquots we evaluated the cell viability (by Trypan Blue exclusion test counting at least 500 cells) with microscopic examination of labelled cells. Fluorescence activated cell sorting (FACS) analysis was performed to count different blood cell subsets and platelets present per mm^3 of the final product and sterility of labelled cells was assessed by haemoculture.

All previous experiments were duplicated and in addition LE was also evaluated in Leukokit® in plasma vs the standard procedure in PBS to verify the influence of washing medium on the LE based on previously obtained results with the prototype.

Using commercially available Leukokit®, we first tested the biocompatibility of all components, and again the LE, erythrocyte sedimentation speed, sterility, apyrogenicity and cell viability. ACD-A, HES and PBS provided with Leukokit® were also compared to Heparin, Dextran and autologous plasma, respectively. In 4 samples, we tested the chemotactic activity of purified WBC against 1 mg/mL of lipopolysaccharide (LPS) and chemotaxis of ^{99m}Tc-HMPAO-labelled WBC (925 MBq, 25 mCi) was compared to that of unlabelled cells. For the multi-centre study, 70 labelling procedures were performed with the Leukokit® by 9 expert operators and 3 beginners from five centers using blood from both patients and volunteers. Finally, Media-Fill tests were performed by 3 operators on two different days (11 procedures) by replacing blood and kit reagents with bacterial culture media (Tryptic Soy Broth) and testing sterility of aliquots of the medium at the end of procedure.

Leukocyte purification using the Leukokit® device kit

All steps were carried out in the laboratory (non-classified room) in non-sterile conditions on the bench, but using the sterile disposables and vials provided. The operators wore latex gloves during the entire procedure and all entries through the entrance rubber septum and to the plastic needle where preceded by an alcohol wipe. All additions to the separation vial were done with a needle fitted syringe through the entrance rubber septum of the injection port. All withdrawals from the device were done through a syringe connected to the plastic needle fitted in the device through the one-way sealing-valve. Peripheral blood, 45 mL, was withdrawn from an arm vein using a 60 ml syringe fitted with a 19G-butterfly and containing 5 mL of anticoagulant (ACD) from the ACD vial. Blood was gently mixed with ACD during withdrawal. Immediately after blood withdrawal, 7.5 mL of sedimentation reagent (10% HES) was aspirated from the HES vial into the syringe with the blood-ACD mixture. Blood was left in the syringe to settle for 60 minutes at room temperature at a 45° inclination. After the red blood

cells were settled, the needle was changed and the cell-rich plasma was transferred into the separation vial by gently pushing the syringe piston, avoiding red cells contamination. The separation vial was then centrifuged for 10 minutes at 150 g. After centrifugation, the leukocyte pellet is visible at the bottom of the separation vial. The supernatant was carefully removed from the vial, avoiding resuspension of the pellet. The leukocyte pellet was then resuspended in 0.5 mL of PBS by gently swirling the vial.

Preparation of ^{99m}Tc-HMPAO

HMPAO (GE Healthcare) was reconstituted with 4 ml (5920 MBq, 160 mCi) of freshly eluted ^{99m}TcO4- from a 0.56 TBq (15.13 Ci) Tc-generator (GE Healthcare). For each labelling quality control was performed by adding few drops of ^{99m}Tc-HMPAO to a mixture of ethyl acetate and 0.9% NaCl (1:1). The ethyl acetate/0.9% NaCl mixture was thoroughly shaken and the two layers, upper lipophilic and lower hydrophilic, were allowed to separate. Samples from both the layers were counted separately to evaluate the percentage of lypophilic ^{99m}Tc-HMPAO and hydrophilic ^{99m}Tc-HMPAO.

Leukocyte labelling with ^{99m}Tc-HMPAO using Leukokit®

Within 15 minutes from reconstitution, 0.5 mL ^{99m}Tc-HMPAO was added to the cells in the separation vial and left to incubate for 10 minutes at room temperature. At the end of incubation, 3 mL of PBS was added to stop the labelling reaction and the separation vial was centrifuged for 5 minutes at 150 g. The supernatant was carefully removed to avoid resuspending the cell pellet. The supernatant was retained to calculate the LE. Finally, 3 mL of PBS was added to the separation vial and the cells were gently resuspended. At the end of the labelling procedure, the LE was calculated using a gamma counter according to the following formula:

LE (%) = [radioactivity bound to cells] / [radioactivity bound to cells + radioactivity in supernatant] x 100.

Sedimentation capacity of HES

Because different red blood cell sedimentation agents are used in WBC labelling, we compared erythrocyte sedimentation rate (ESR) of 10 blood samples with 10% HES (Fresenius, France), with 6% HES (Fresenius, France) and without sedimentation agent (control) at 30, 60, 90 and 120 min. For the experiments, 0.5 mL of 10% HES or 6% HES was added to 4.5 mL of blood from normal volunteers and erythrocyte sedimentation was compared with that of blood samples from the same volunteers but without HES.

Sterility tests

The presence of Gram-positive and Gram-negative bacteria was tested in the following solutions: ACD, HES and PBS before and after being inserted in the device. All experiments were performed in triplicates with 3 random chosen vials of each reagent. Sterility was also tested in the samples of radiolabelled cells (after the whole procedure using the device kit and all reagents and disposables) from 10 normal volunteers. In 11 procedures by 3 operators on two different days, Media-Fill tests were performed. These consisted in replacing blood and kit reagents with bacterial culture media (Tryptic Soy Broth, TSB) and testing sterility of TSB aliquots at the end of the procedure.

Pyrogenicity tests

The presence of pyrogens has been evaluated by the Limulus Amebocyte Lysate (LAL) test as described in the guidelines by the FDA. This test is an enzyme-mediated assay for the detection of endotoxin activity and is internationally established and delivers reliable results.[11] The LAL test was performed on the following solutions: ACD, HES, PBS and PBS after being inserted in the device. All experiments were performed in triplicates with 3 random chosen vials of each reagent.

Toxicity tests on labelled white blood cells

Possible toxicity induced by the components of the device kit has been evaluated by testing the viability (Trypan blue exclusion test and clumping) and functionality of white blood cells (chemotaxis). Purified white blood cells obtained from 5 normal volunteers were resuspended in Roswell Park Memorial Institute (RPMI) culture medium and inserted in the separation vial, centrifuged twice at 180 g for 10 min-

utes and resuspended each time with sterile RPMI. As control, purified leukocytes were centrifuged in Falcon vials. These experiments were planned to verify if the plastic hardware of the device could activate or damage the cells. Furthermore, the test was also performed on leukocytes from the same 5 normal volunteers as above but purified, washed and labelled with ^{99m}Tc-HMPAO using all reagents and disposables provided with the device kit, to verify cell function at the end of the complete cycle.

The chemotactic activity against 1 mg/ml of LPS was tested in 4 samples of purified WBC. In addition, control experiments were performed for several parameters. Thus, WBC were purified using heparin instead of ACD, or Dextran instead of HES, or autologous plasma instead of PBS as the medium for washing. Furthermore, the chemotaxis of leukocytes labelled with ^{99m}Tc-HMPAO using the device kit was compared with that of unlabelled cells and cells labelled by the standard technique.

The viability of the labeled WBC was determined at the end of labelling procedure by the Trypan Blue exclusion test. Thus, an equal volume of 0.4% Trypan blue solution in water was added to a sample of the labelled leukocyte cell suspension and gently mixed. A drop of the cell suspension was pipetted in a haemocytometer. The haemocytometer was placed under a phase-contrast microscope at 100-fold magnification. The counting chamber was checked for clumps and the percentage of blue-stained cells (cells that have been damaged during the labelling process) was counted.

Multi-centre study on the routine use of labelling procedure with Leukokit®

All people that performed the labelling procedure (9 expert operators and 3 beginners, from the following centers: Nuclear Medicine Unit, Faculty of Medicine and Psychology, "Sapienza" University, Roma, Italy; Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, the Netherlands; Regional Center of Nuclear Medicine, University of Pisa, Italy; Nuclear Medicine, Azienda Ospedaliero-Universitaria Ferrara, Italy; Nuclear Medicine, S. Croce e Carle Hospital, Cuneo, Italy) were asked to fill in a questionnaire of 40 questions. They had to record the total procedure time and the sedimentation time and they had to score the easiness or difficulty of all steps in the procedure and the easiness of the whole procedure (7 scores: very easy, easy, average, need to be performed by experienced personnel, a little complicated, difficult or very difficult). If an operator performed more labelling procedures, the learning curve was determined.

Results

Results of the experiments performed with the second prototype and the Leukokit®

The LE was calculated in blood from 7 volunteers with the second prototype in PBS and was compared to the standard procedure in plasma (Figure 3). The mean LE with the second prototype in PBS was 81.8 ± 3.0 and with the standard procedure in plasma 64.6±5.4 (P=0.0003).

To verify the role of washing medium in the LE

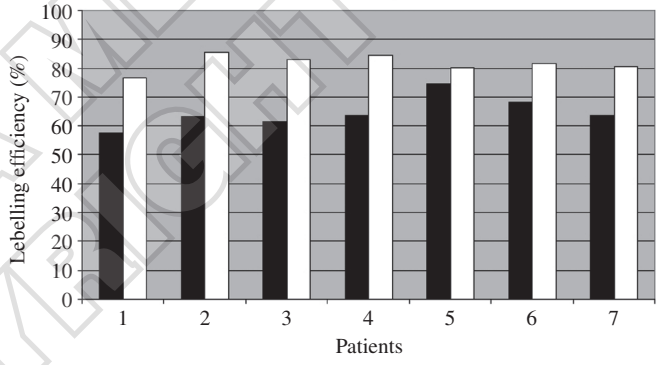

Figure 3.—Labelling efficiency with the standard technique in plasma (dark bars) compared to the second prototype in PBS (light bars).

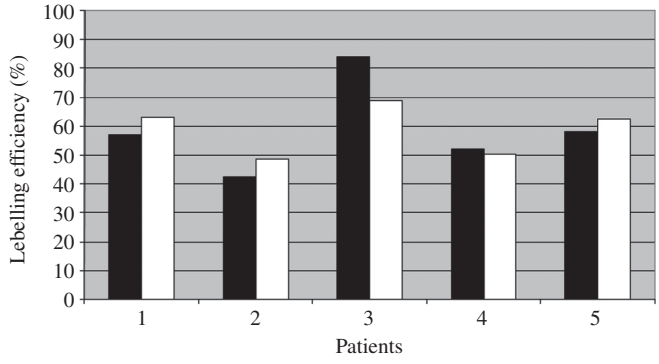

Figure 4.—Labelling efficiency with the standard technique in PBS (dark bars) compared to the Leukokit® in plasma (light bars).

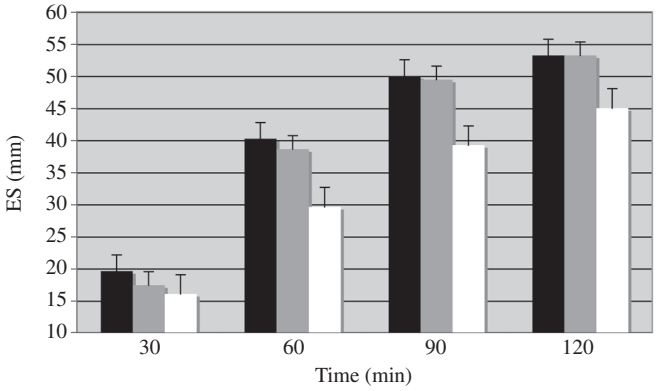

Figure 5.—Mean of erythrocyte sedimentation distance±SD (ES) of ten blood sedimentation procedures after the use of HES 10% (black bars), HES 6% (gray bars) and without ESR reagent (white bars). All differences are statistically non significant.

tests of the second prototype this test was again evaluated with Leukokit® in 5 volunteers but now in plasma and compared to the standard procedure in PBS (Figure 4). The LE was comparable (58.8±7.81 with Leukokit® in plasma *vs.* 57.36 ± 14.26 with the standard procedure in PBS).

All biocompatibility tests according to the appropriate UNI-EN-ISO-10993 and European Pharmaceutical Guidelines showed normal findings, considering all materials and products used in the device kit biocompatible.

In 5 volunteers, the LE in the Leukokit® procedure was compared to the ISORBE consensus protocol. The LE in the Leukokit® procedure was significantly higher as compared to the standard technique, as previously demonstrated using the prototype (80±3.2% *vs.* 58±6%, P<0.001).

The overall erythrocyte sedimentation time was between 40 and 60 minutes. The ESR was compared in blood of 4 persons with 10% HES, with 6% HES and without sedimentation agent (control). Both 10% HES and 6% HES simulated ESR, but 10% HES showed a greater improvement in ES than 6% HES (Figure 5).

In vitro tests confirmed the full sterility (heamoculture) and apyrogenicity (LAL test) of all materials and solutions included in the Leukokit® device. After labelling with the Leukokit® procedure, suspensions of labelled WBC were also shown to be sterile. The 11 Media-Fill tests were all sterile.

The average white blood cell recovery (in 4 samples) was $117x10^6$±$24x10^6$ using the Leukokit® procedure and $132x10^6$±$29x10^6$ using the standard ISORBE technique (two-sided paired Students' t-test, P=not significant). There was also no significant difference in these 4 samples in red blood cell, neutrophil, lymphocyte and platelet content (Table I).

The viability of the cells after labelling with the Leukokit® procedure was 99.9%, as was determined by the Trypan blue exclusion test.

None of the chemotactic tests showed any significant difference between Leukokit® samples and controls. First we tested the chemotaxis of WBC with Leukokit® (with HES as sedimentation agent) and compared it to the standard technique (with Dextran as sedimentation agent). As shown in Table II (Donor A and B) no significant difference in

TABLE I.—*Erythrocyte, neutrophil, lymphocyte and platelet content in the purified WBC obtained with the Leukokit® procedure and ISORBE consensus protocol (Control). No significant differences between the procedures were observed (paired t-test).*

	Erythrocytes (10^9/mL)		Neutrophils (10^7/mL)		Lymphocytes (10^7/mL)		Platelets (10^4/mL)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Leukokit®	0.030	0.01	7.595	1.537	3.548	1.203	63.000	11.605
Control	0.025	0.01	8.083	2.087	4.163	1.328	59.250	8.995
Paired *t*-test	ns		ns		ns		ns	

TABLE II.—*Results of chemotactic tests of WBC, comparing Leukokit® procedure (with HES) to standard technique (Control, with Destran).*

	WBC Leukokit®	WBC Control
Donor	HES (µmol/L)	Destran (µmoL/L)
A	109.3 ± 1.5	114.2 ± 2.0
B	104.0 ± 5.6	105.5 ± 2.1

TABLE III.—*Results of chemotactic tests of WBC. comparing Leukokit® procedure with PBS to control group with autologous plasma. Both procedures were tested with HES and with Destran.*

Donor	WBC Leukokit® + PBS		WBC Control + Plasma	
	HES (µmol/L)	Destran (µmol/L)	HES (µmol/L)	Destran (µmol/L)
C	107.0 ± 3.2	113.0 ± 2.1	124.0 ± 3.4	111.0 ± 2.0
D	52.5 ± 1.4	61.5 ± 3.2	47.3 ± 3.5	46.5 ± 2.6

TABLE IV.—*Results of chemotactic test of unlabelled WBC, compared to WBC labelled with Leukokit® procedure and ISORBE consensus protocol (Control).*

Donor	Unlabelled WBC (µmol/L)	^{99m}Tc-WBC Leukokit® (µmol/L)	^{99m}Tc-WBC Control (µmol/L)
E	106.0 ± 2.2	101.0 ± 2.3	104.0 ± 1.4

chemotaxis between these labelling methods was seen. After that, we tested the chemotactic activity of WBC washed with PBS and compared it to a control group with WBC washed with autologous plasma. As erythrocyte sedimentation reagent, we used both HES and Dextran. No significant differences were seen between PBS and autologous plasma or between HES and Dextran (Table III, Donor C and D).

Finally, we compared the chemotactic activity of unlabelled WBC with that of WBC labelled with the Leukokit® procedure and with the standard labelling technique. Again, no significant differences in chemotaxis were seen (Table IV, Donor E).

In general, expert operators as well as beginners accepted the Leukokit® procedure very well and scored the procedure as easy or average. Some operators indicated after the first time they performed the procedure, it needed to be performed by experienced personnel only, but their confidence grew after more tests. The learning curve was very fast and experts and beginners had confidence in the whole procedure after 2±2 tests.

Discussion

WBC labelling requires isolation of cells from patient's blood under sterile conditions using sterile materials, buffers and disposables. Therefore, the use of WBC-S is limited to well equipped laboratories and trained personnel. We developed and tested a disposable, sterile, closed device (called Leukokit®) that allows blood manipulation, WBC purification and labelling without exposing patient's blood and operator to contamination risks. A laminar flow hood cabinet appears to be not required if not in contrast with the legal recommendations of the country, and personnel can be very easily trained in the use of Leukokit®. In each device kit, GMP aliquots of HES, ACD and PBS are included. All plastic components were tested for biocompatibility according to the appropriate UNI-EN-ISO-10993 guideline.

All *in vitro* tests confirmed full biocompatibility, sterility and safety of materials and reagents. Haemocultures and Media-Fill tests were always sterile. Compared to the standardized technique, the ISORBE consensus protocol, the average WBC recovery was comparable, as was the red blood cell, the lymphocyte and platelet content. The labelling efficiency with Leukokit® was even significantly higher compared to the consensus protocol in plasma and this seems to be related mainly to the use of PBS as washing medium instead of plasma. Chemotactic tests showed no differences at all between all Leukokit® samples and controls, including plasma vs PBS. The procedure was very well accepted by experts and also by beginners with a very fast learning curve. Leukokit® represents a single-patient disposable sterile kit for WBC labelling with radioisotopes. More than one labelling procedure per day is also possible due to the different colour codes, which are also included in the kit. Each patient has his or her own colour, which decreases the risk for patient contamination and reinjection in the wrong patient.

For the future, maybe this device could also be changed and tested for labelling red blood cells and platelets as for other cell labelling procedures.

Conclusions

We conclude that the Leukokit® device offers a disposable sterile kit for WBC labelling with radioisotopes which is easier to use compared to the standard techniques and will make WBC labelling available for more clinical centers and more patients.

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